

## Zombie Red™ Fixable Viability Kit

<b>Catalog# / Size</b>	423109 / 100 tests 423110 / 500 tests
<b>Regulatory Status</b>	RUO
<b>Other Names</b>	Fixable Dye, Fixable Viability Dye
<b>Description</b>	Zombie Red™ is an amine reactive fluorescent dye that is non-permeant to live cells, but permeant to the cells with compromised membranes. Thus, it can be used to assess live vs. dead status of mammalian cells. Zombie Red™ is a polar water soluble dye, providing red fluorescence, making it suitable for multi-color detection.

### Product Details

---

<b>Preparation</b>	Zombie Red™ Fixable Viability kit is composed of lyophilized Zombie Red™ dye and anhydrous DMSO. For reconstitution, bring the kit to room temperature; add 100 µl of DMSO to one vial of Zombie Red™ dye until fully dissolved. 100 tests = 1 vial of Zombie Red™ + DMSO, 500 tests = 5 vials of Zombie Red™ + DMSO.
<b>Storage &amp; Handling</b>	Store kit at -20°C upon receipt. Do not open vials until needed. Once the DMSO is added to the Zombie Red™ dye, use immediately, or store at -20°C in a dry place and protected from light, preferably in a desiccator or in a container with desiccant for no more than one month.
<b>Application</b>	<a href="#">FC, ICFC - Quality tested</a> <a href="#">ICC - Verified</a>
<b>Recommended Usage</b>	Each lot of this product is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometry, the suggested dilution is 1:100-1:1000 for 1-10 million cells. For immunocytochemistry, the suggested dilution is 1:1000. It is recommended that the reagent be titrated for optimal performance for each application, as optimal dosage varies with cell type.
<b>Excitation Laser</b>	Blue Laser (488 nm) Green Laser (532 nm)/Yellow-Green Laser (561 nm)
<b>Application Notes</b>	<p><b>Standard Cell Staining Protocol:</b></p> <ol style="list-style-type: none"><li>1. Prior to reconstitution, spin down the vial of lyophilized reagent in a microcentrifuge to ensure the reagent is at the bottom of the vial.</li><li>2. For reconstitution, pre-warm the kit to room temperature; add 100 µl of DMSO to one vial of Zombie Red™ dye and mix until fully dissolved</li><li>3. Wash cells with PBS buffer (no Tris buffer and protein free).</li><li>4. Dilute Zombie Red™ dye at 1:100-1000 in PBS. Resuspend 1-10 x 10<sup>6</sup> cells in diluted 100 µl Zombie Red™ solution. To minimize background staining of live cells, titrate the amount of dye and/or number of cells per 100 µl for optimal performance. Different cell types can have a wide degree of variability in staining based on cell size and degree of cell death.</li></ol> <p><b>Note:</b> Don't use Tris buffer as a diluent and be sure that the PBS does not contain any other protein like BSA or FBS. <b>Note:</b> The amount of dye used can also influence the ability to detect apoptotic as well as live and dead cells.</p> <ol style="list-style-type: none"><li>5. Incubate the cells at room temperature, in the dark, for 15-30 minutes.</li><li>6. Wash one time with 2 ml BioLegend's Cell Staining Buffer (Cat. No. 420201) or equivalent buffer containing serum or BSA.</li><li>7. Continue performing antibody staining procedure as desired.</li><li>8. Cells can be fixed with paraformaldehyde or methanol prior to permeabilization or can be analyzed without fixation.</li></ol> <p><b>No-wash Sequential Staining Protocol:</b></p> <ol style="list-style-type: none"><li>1. Wash cells with PBS buffer (no Tris buffer and protein free).</li><li>2. For reconstitution, pre-warm the kit to room temperature; add 100 µl of DMSO to one vial of Zombie Red™ dye and mix until fully dissolved</li><li>3. Determine the total µl volume of antibody cocktail previously titrated and optimized for the assay</li></ol>

that will be added to each vial/well of cells based on a final volume of 100  $\mu$ l. Subtract that antibody volume from the 100  $\mu$ l total staining volume intended for the assay. In the remaining volume, dilute Zombie Red™ dye at 1:100-1000 in PBS as determined by prior optimization at that volume. For example, if you are adding 20  $\mu$ l of antibody cocktail for a 100  $\mu$ l total staining volume, use 80  $\mu$ l of Zombie Red™ solution. Resuspend 1-10  $\times 10^6$  cells in the appropriate volume of Zombie Red™ solution. Different cell types can have a wide degree of variability in staining based on cell size and degree of cell death.

**Note:** Don't use Tris buffer as a diluent and be sure that the PBS does not contain any other protein like BSA or FBS.

**Note:** The amount of dye used can also influence the ability to detect apoptotic as well as live and dead cells.

4. Incubate for 10-15 minutes at RT, protected from light. Without washing the cells, add the cell surface antibody cocktail and incubate for another 15-20 minutes.
5. Add 1-2 mL Cell Staining Buffer (Cat. No. 420201) or equivalent buffer containing BSA or serum. Centrifuge to pellet.
6. Continue with normal fixation and permeabilization procedure. If planning to skip fixation and analyze cells live, complete an additional wash step to minimize any unnecessary background of the live cells.

**Notes:** If the cell type in use cannot tolerate a protein-free environment, then titrate the Zombie Red™ dye in the presence of the same amount of BSA/serum as will be present in the antibody staining procedure. A higher amount of Zombie Red™ may be required since the BSA/serum will react with and bind up some proportion of the Zombie Red™.

Zombie Red™ dye is excited by the yellow/green laser (561 nm) and has fluorescence emission maximum at 624 nm. If using in a multi-color panel design, filter optimization may be required depending on other fluorophores used. Zombie Red™ dye has similar emission to PE-Texas Red.

Zombie Red™ can be weakly excited by the 488 laser, if a yellow/green laser is not available. This may require a higher concentration of Zombie Red™ dye; optimization by the end user would be required.

## Product Citations

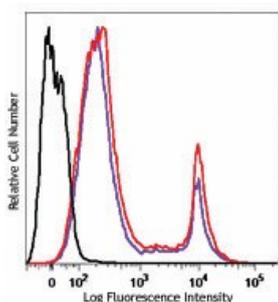
1. Mercer F, *et al.* 2016. PLoS Negl Trop Dis. 10: 0004913. [PubMed](#)
2. Martin C, *et al.* 2017. PLoS One. 10.1371/journal.pone.0170504. [PubMed](#)
3. Antsiferova M, *et al.* 2017. EMBO Mol Med. 9(1):27-45. [PubMed](#)
4. Holokai L, *et al.* 2020. Cancers (Basel). 12:00. [PubMed](#)
5. Krausgruber T, *et al.* 2020. Nature. 583:296. [PubMed](#)
6. Lindner SE, *et al.* 2020. Immunohorizons. 0.356944444. [PubMed](#)
7. Feng J, *et al.* 2017. Nat Commun. . 10.1038/s41467-017-01056-8. [PubMed](#)
8. Riopel M, *et al.* 2019. Mol Metab. 20:89. [PubMed](#)
9. Sperber HS, *et al.* 2019. Sci Rep. 9:707. [PubMed](#)
10. Yen WF *et al.* 2019. Cell reports. 27(5):1472-1486 . [PubMed](#)
11. Brennan FH, *et al.* 2019. JCI Insight. 4. [PubMed](#)
12. Haertel E, *et al.* 2018. Eur J Immunol. 48:1001. [PubMed](#)
13. Mousseau G, *et al.* 2019. MBio. 10. [PubMed](#)
14. Ligorio M, *et al.* 2019. Cell. 178:160. [PubMed](#)

## Antigen Details

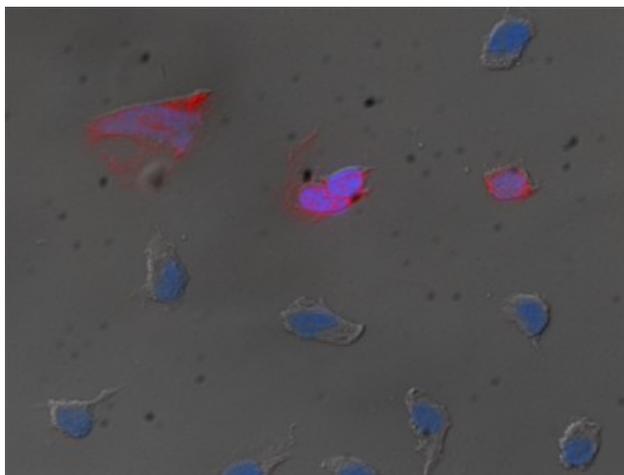
**Biology Area** Apoptosis/Tumor Suppressors/Cell Death, Cell Biology, Neuroscience

**Gene ID** NA

## Product Data



One day old C57BL/6 mouse splenocytes were stained with Zombie Red™ and analyzed before fixation (purple) or after fixation and permeabilization (red). Cells alone, without Zombie Red™ staining, are indicated in black.



HeLa cells were treated with 20% EtOH for 20 seconds, washed twice with PBS, and then were left to recover for five minutes with cell culture media in 37°C. The cells were stained with Zombie Red™ (1:1000) (red) for 15 minutes and then fixed with 1% paraformaldehyde (PFA) for ten minutes. Nuclei were counterstained with DAPI (blue) for five minutes. The image was captured with 40X objective.

For research use only. Not for diagnostic use. Not for resale. BioLegend will not be held responsible for patent infringement or other violations that may occur with the use of our products.

\*These products may be covered by one or more Limited Use Label Licenses (see the BioLegend Catalog or our website, [www.biolegend.com/ordering#license](http://www.biolegend.com/ordering#license)). BioLegend products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products, reverse engineer functionally similar materials, or to provide a service to third parties without written approval of BioLegend. By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 [www.biolegend.com](http://www.biolegend.com)  
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587